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Mycoflora of Well Water in the Dairy Farms of the Town of Hatillo Puerto Rico

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Abstract:
Water wells are one of the main sources of supply of this resource in dairy farms. In P.R. these waters are used in the dairy farms, but when there is an atmospheric phenomenon such as a hurricane, people consume it, because there is no electrical service. Organisms that have a low content of nutrients live in these waters. But with agricultural activities and anthropogenic contamination, they are the main factors of contamination of aquifers. The objective of this study is to make an inventory of filamentous fungi and yeasts in 3 dairy farms water wells. Monthly samples were taken for a whole year. The membrane filtration method was used. The culture media used were RBA, SDA and PDA in triplicate. The isolates were identified using morphological and microscopic characteristics and for yeasts the RapID Yeast Plus ™ test was used. All the wells presented contamination by fungal growth. The water well with the highest growth was well A. The months with the highest growth of colonies found were December and January. Seven genus and 18 species of fungi and yeasts were isolated. The genus found were *Aspergillus, Rhizopus, Candida, Penicillium, Curvularia, Trichosporon* and *Neoscytalidium*. The species found were: *A. nidulans, A. oryzae, A. terreus, A. niger, A. flavus, A. ochraceus, A. tamarii, A. ustus, R. stolonifer, C. tropicalis, C. rugosa, C. parapsilosis, C. rugosa, C. purpurogenum, P. chrysogenum, P. expansum, Curvularia hominis,* and *T. beigelii*. The temperature was 22.1 °C to 29.6 °C, while the pH was 7.62 to 8.66. The presence of fungi may indicate that there is contamination of anthropogenic origin in the water wells, of each dairy farm. Many of these fungal species are associated with various infections in humans and animals. We can point out the importance of disinfection in those water wells that will be used as drinking water by humans and animals.

INTRODUCTION

Some of the important sources of water consumption are water wells. In Puerto Rico we find it in the North area. The town of Hatillo in Puerto Rico historically depends on agriculture, and above all on dairy farms for work (Guzmán, 2014). The dairy farms produce a large amount of waste, which is stored in oxidation ponds. These wastes are used as fertilizers through various methods, causing them to fall to the ground daily (Du, 2012).

The Northwestern area of the island is characterized by having limestone plains and alluvial soils. These are ideal for working in the agricultural sector. The northern karstic zone has the largest aquifer in Puerto Rico. The dairy farms use this groundwater for the consumption of the population as well as for their animals (Fosberg *et al.,* 2014).

Lugo et al. (2004) indicate that the karst belt of Puerto Rico represents one of the least impacted karst sites in the Caribbean area. But, on the other hand, the limestone region is very sensitive to human activities. This includes the clearing of vegetation, the paving of wooded areas, filling of wetlands, contamination from the misuse of the soil, the excessive use of aquifers and the contamination of groundwater.

All these problems of contamination of water resources can also be observed worldwide as well. In Slovenia a study identified the fungi present in 100 tap water and 16 ground waters well samples. They found 28 species of fungi and 16 genus. Among those were *Candida parapsilosis*, *Aspergillus sp.* and *Exophiala sp.* (Novak Babič *et al.,* 2016). On the other hand, in the Zacatepec aquifers, in Mexico, another study measured the physical-chemical parameters of the water for one year, finding that the pH obtained was between 6.7 and 7.3, and the temperature was between 25.1 °C and 28 °C. Therefore, no temporal variations were observed in the physicochemical parameters (Ramírez, 2009).

In Costa Rica, a mycological characterization study of raw and filtered water from treatment plants in three rivers was carried out. They used the membrane filtration method and found that 63% were filamentous fungi and 37% were yeasts, corresponding to 22 genus. One of the most found genus was *Candida* (19%). Among the filamentous fungi that they isolated they found; *Geotrichum candidum, Rhodotorula, Saccharomyces* and *Trichosporon sp*. Most of the filamentous fungi were eliminated in the filtration process. This may be a common situation in tropical countries and represents a special health risk for immunocompromised patients (Valiente, 1993).

In municipal waters in Estanbul, Turkey a study report that they found 51 CFU/100ml in hospital samples 23 CFU/100ml in household waters and 98 CFU/100ml in different waters. Among the fungal species identified were *F. oxysporum, Aspergillus flavus, Alternaria, Aspergillus clavatus, A. fumigatus* and *Cladosporium cladosporioides*. This study demonstrated the presence of mycotoxigenic fungi in the municipal water supply. Therefore, it indicates that disinfection procedures may be insufficient (Göksay and Demirel, 2018).

In a groundwater investigation carried out in Brazil, the results obtained showed that between 5 to 207 CFU/100ml with a mean value of 53 CFU/100ml. The most representative genus is *Aspergillus* with 37% and *Penicillium* with 25%, *Trichoderma* and *Fusarium* with 9%, *Curvularia* with 5% and *Pestalotiopsis karstenii* with 21%. They also isolated yeasts such as *Ramichloridium* and *Leptodontium*. Therefore, they consider that the system can play an important role in the spread of fungi, including opportunistic pathogenic fungi (Olivera *et al.,* 2016).

Filamentous fungal species such as *Aspergillus sp*., *Penicillium sp*., *Fusarium sp*., and *Mucor sp.* were found in groundwater wells in Giza, Egypt, and some yeast species, such as *Saccharomyces cerevisiae* and *Candida*. Therefore, the study concludes that groundwater could be considered as a possible route of transmission of filamentous fungi and yeasts, which can constitute a potential problem for public health (Mohamed *et al.,* 2014).

In another study by Nwankwo 2020, they identified 16 isolates, belonging to 6 genus, which included: *Acremonium sp., Aspergillus sp., Candida sp., Fusarium spp., Penicillium* and *Phialophora sp., Candida sp*., which obtained the highest amount, with 43.75%, followed by *Fusarium sp*., *Acremonium sp*., and *Phialophora sp*. with 6.25% each. These pathogenic fungi in groundwater represent a high and serious public health problem. Monitoring and treatment of the water before being consumed and for any other use was recommended.

In a study carried out by Youssef (2019), he pointed out that the concern of consumers is microbial contamination in water. It was found that the yeast specie *Candida glabrata* was the yeast with the most isolates in surface waters with 60% to 80% in summer. While in groundwater 31% to 41% were identified in summer. The genus with the most species isolated was *Aspergillus*, of which the second was *A. flavus* and the third *was A. niger*.

In Sa Jose Do Rio Preto, São Paulo, Brazil, their study indicates that fungal contamination was 78.8%, and yeast species were also identified. The study indicates that fungi can grow even within recommended standards including chlorine and pH parameters (Gazzola *et al.,* 2019). Some isolated species in the registered water well were; *A. fumigatus, Penicillioides, Japonicus, Marneffei caecilius, Flavus, Penicillium marneffei, Marquandii, Expansus, Acremonium hyalinulum, Curvularia clavata, Fusarium incarnatum, Candida guilliermondii, Candida parapsilosis, Blastoschizomyces capitatus*, and *Aureobasidium pullulans*. 80% of the samples had fungal contamination in the non-registered water well. Some of these species were; *A. fumigatus, Penicillioides, Japonicus, Flavus, Clavatus, P. commune, P. decumbens, P. expansum, P. citrinum, P. spinlosum, P. purpurogenum, Fusarium incarnatum, Basidiobolus ranarum, Acremonium hylinulum, Scytalidium hyalinum*, and *Curularia clavata*. Some species of yeasts identified were; *Aureobasidium pullulans, Candida guilliermondii, Candida intermedia, Trichosporon asahii, Candida tropicalis, Candida glabrata, Candida lusitaniae, Candida parapsilosis, Trichosporon mucoides, Candida fomata, Candida silvivola, Kodomaea ohmeria, Rhodotarula minuta, Rhodotorula glutinis*, and *Zygosaccharomyces florentinus* (Gazzola *et al.,* 2019).

There is a lot of interest in studying and monitoring the potential impacts of yeasts in aquatic environments. Many of these identified species are pathogenic and antifungal. It is necessary to study these ecosystems to compare and analyze the possible effects on public health in the country. Most studies of aquatic yeasts have linked them to water contamination. Yeasts cause a variety of diseases, some of these yeast species are resistant to veterinary antifungal agents (Monapathi *et al.,* 2020). The *C. parapsilosis* species, which is an opportunistic pathogen, has been found in tap water, bathroom, washing machines, kitchen surfaces, dishwasher and refrigerator samples (Zupancic *et al.,* 2018).

The fungi found in the water cause problems since they alter the taste, smell and cause various diseases to humans and animals. There are no regulations that cover the problems caused by fungi in aquatic environments. Filamentous fungi in a drinking water distribution system can cause pipe blockages, organoleptic biodeterioration, health problems as pathogens or allergens, and can cause mycotoxin contamination (Novak Babič *et al.,* 2016).

The weather will probably also be important to the frequency and growth of fungi because they depend on the weather seasons. If the weather has snow, once it melts, drags the water into rivers, lakes and groundwater, so fungi are present everywhere. Therefore, monitoring the number of fungal species is important (Hageskal *et al.,* 2006).

Groundwater is a source of drinking water, but fungal contamination from anthropogenic activities and runoff infiltration into groundwater results in being unfit for human consumption and other recreational purposes. Groundwater can be contaminated chemically, physically and microbiologically, so they can have various reasons related to various sources of contamination and therefore increase health problems (Nwankwo *et al.,* 2020).

Studies detect a higher frequency of species, depending on the morphology and other phenotypic characters. Which showed that the fungi occur widely in drinking water sources, so therefore it is recommend further studies to see their potential for biodegradation, as well as improving the filtration processes in drinking water that are currently used. In this way, it is sought to be able to analyze if these methods are effective and if there are any side effects (Pereira *et al.,* 2009).

MATERIALS AND METHODS

Three water wells in the town of Hatillo, Puerto Rico were studied for one year. Samples were collected once a month. The sampling points of the dairy water wells were identified with the letters A, B and C. Once the sample is taken, the pH and temperature parameters will be taken from each well water sample (Bello and Echevarría, 2022). The samples were collected in sterilized bottles. They were transported in a container with ice to the laboratory. The 0.45µm pore size membrane filtration method was used (Baird *et al.,* 2017). 100 ml of water sample from each well was filtered. The filtrate was placed in the culture dish in the different culture media, in triplicate (Al-gabr *et al.,* 2014). The culture media used were; Sabouraud Dextrose agar (SDA), Potato Dextrose agar (PDA) and Rose Bengal agar (RBA). To carry out the positive control, the dishes were inoculated with each medium (SDA, PDA and RBA) with *Aspergillus fumigatus*. The negative control was one plate of each uninoculated medium. The incubation period of the samples was 7 to 14 days at 25°C (Echevarría, 2019a,b; 2022). Then the colonies were counted, to obtain the averages of (CFU) and in each culture medium the isolates were made (De Hoog *et al.,* 2004). Identification was made with macroscopic and microscopic identification. They were observed under the Nikon Eclipse Ci microscope. The data obtained were compared using taxonomic keys. Yeast samples were identified through the RapID Yeast Plus ™.

RESULTS AND DISCUSSION

In one year of sampling, 7 genus and 18 species of filamentous fungi and yeasts were isolated in the water of the 3 wells in the dairy farms in the town of Hatillo. The most frequently isolated genus in the three wells were; *Aspergillus and Candida*. In table 1 we observe the genus and species of each isolate from well A, B, and C in the months of April 2018 to April 2019. Well A obtained a total of 48 isolates, well B obtained 46 and well C obtained 56 in total of all sampling months.

The species isolated in well A were; *C. rugosa, A. terreus, A. flavus, C. parapsilosis, A. nidulans, A. ochraceus, A. niger, A. oryzae, Neoscytalidium, R. stolonifer, C. tropicalis, P. chrysogenum, T. beigelii, P. expansum, A. ustus* and *P. purpurogenum*. The species isolated from well B were; *A. oryzae, A. terreus, A. flavus, C. parapsilosis, A. nidulans, R. stolonifer, C. rugosa, A. nidulans, C. tropicalis, A. ochraceus, P. purpurogenum, Trichosporon beigelii,* and *A. niger*. The species isolated from well C were; *A. terreus, A. flavus, A. oryzae, C. parapsilosis, A. niger, Curvularia hominis, C. tropicalis, R. stolonifer, P. purpurogenum, C. rugosa, A. ochraceus, P. expansum* and *A. tamarii*.

Table 2 shows a summary of the species isolated in this study, showing their pathogenicity or ecology. Most of these fungi identified in this study cause a variety of infections (Berger, 2015). Yeasts were observed and isolated in all water samples from wells in the study, except well B in the months of May, June, August, November and March. Also, in well A they were not found in the months of May, August, September 2018, and April 2019. Well C only obtained isolates in August 2018, therefore, diversity of *Candida* species was found in all the samples. The isolated yeasts were *Candida parapsilosis, Candida rugosa* and *Candida tropicalis*.

As part of the analysis, we worked every month with two controls: one negative and one positive. The negative control was used to demonstrate that the culture medium was sterile and had no growth. In the case of the positive control, it was inoculated with *Aspergillus fumigatus*, this to guarantee that the medium reproduces and is suitable for the growth of the samples. In all months the expected results were obtained; negative controls had no growth and positive controls had growth.

Table 1. Total Genus and species of fungi identified by month in the water wells of three dairy wells in Hatillo P.R.

Date	Well	Fungi and yeast	total
April 2018	A	*C. rugosa, A. terreus, A. flavus, C. parapsilosis, A. nidulans, A. ochraceus, A. niger*	7
	B	*A. oryzae, A. terreus, A. flavus, C. parapsilosis, A. nidulans*	5
	C	*A. terreus, A. flavus, A. oryzae, C. parapsilosis*	4
May 2018	A	*A. niger, A. terreus, A. oryzae, C. parapsilosis, C. tropicalis, C. rugosa*	6
	B	*R. stolonifer, A. nidulans, A. flavus, A. tererus*	4
	C	*A. niger, A. oryzae, Curvularia huminis, C. tropicalis, C. parapsilosis, A. terreus*	6
June 2018	A	*A. flavus, A. terreus, Neocytalidum*	3
	B	*A. terrus, R. stolonifer*	2
	C	*C. tropicalis, A. oryzae, A. flavus, A. terreus*	4
July 2018	A	*A. terreus, R. stolonifer, A. niger, A. oryzae, A. nidulans, C. tropicalis*	6
	B	*R. stolonifer, C. rugosa, A. nidulans, C. tropicalis*	4
	C	*R. stolonifer, A. terrus, C. parapsilosis, A. oryzae*	4
August 2018	A	*A. oryzae, A. niger*	2
	B	*A. nidulans, A. oryzae, A. niger, A. flavus*	4
	C	*A. oryzae, A. niger*	2
September 2018	A	*A. terreus, A. oryzae, A. niger, A. nidulans*	4
	B	*A. terreus, A. nidulans, C. parapsilosis*	3
	C	*P. purpurogenum, A. niger, C. rugosa, R. stolonifer, C. tropicalis, C. parapsilosis, A. oryzae*	7
October 2018	A	*A. niger, P. chrysogenum, C. tropicalis, A. flavus*	4
	B	*C. rugosa, A. oryzae, A. ochraceus, A. nidulans*	4
	C	*C. rugosa, A. niger, P. purpurogenum, A. oryzae, A. flavus*	5
November 2018	A	*C. rugosa, A. niger*	2
	B	*A. flavus, A. oryzae, P. purpurogenum*	3
	C	*A. ochraceus, C. tropicalis, A. niger, A. flavus, P. purpurogenum*	5
December 2018	A	*P. chrysogenum, Trichosporon beigelii, C. parapsilosis*	3
	B	*C. rugosa, P. purpurogenum, C. tropicalis, Trichosporon beigelii*	4
	C	*C. parapsilosis, A. terreus*	2
January 2019	A	*C. rugosa, C. parapsilosis*	2
	B	*R. stolonifer, C. rugosa, C. parapsilosis, A. terreus*	4
	C	*C. rugosa, A. niger, A. terreus*	3
February 2019	A	*C. rugosa, C. parapsilosis, A. niger*	3
	B	*C. rugosa, A. flavus, A. niger, C. parapsilosis*	4
	C	*C. rugosa, A. flavus, P. purpurogenum, A. oryzae*	4
March 2019	A	*C. rugosa, P. expansum, A. ustus, A. nidulans, C. tropicalis*	5
	B	*A. flavus, P. purpurogenum, A. nidulas, A. oryzae, A. flavus*	5
	C	*A. flavus, P. expansum, C. parapsilosis, P. purpurogenum, A. terreus, A. niger, C. tropicalis*	7
April 2019	A	*P. purpurogenum*	1
	B	*C. rugosa, P. purpurogenum*	2
	C	*A. tamarii, C. rugosa, P. purpurogenum*	3
Dry season (december - april) Wet season (may - november)			

Table 2. Isolated fungi and diseases they cause.

Species	Disease	Reference
C. rugosa	Emerging fungal pathogen, mostly identified in Latin America, presents resistance to antibiotic treatment.	(Pfaller *et al.,* 2006)
A. terreus	Opportunistic fungal pathogen, presents antifungal resistance, adapts to a wide range of environmental conditions.	(Lass-Flörl *et al.,* 2021)
A. flavus	It presents antifungal resistance, a common etiological agent of invasive aspergillosis, predominant in warm environments.	(Rudramurthy *et al.,* 2019)
C. parapsilosis	Human pathogen, causing invasive candidemia and nosocomial infections, *C. parapsilosis* was isolated for the first time from the feces of a patient with diarrhea in Puerto Rico in 1928, it has an extensive distribution in nature, not only in humans.	(Trofa *et al.,* 2008)
A. nidulans	An opportunistic fungal pathogen, it is the main etiological agent of infections in immunocompromised patients and of cutaneous aspergillosis.	(Tavakoli *et al.,* 2020)
A. ochraceus	It acts as a pathogen of humans and animals producing onychomycosis, bronchopulmonary allergic aspergillosis and otomycosis, known to spoil food, beneficial in the production of industrial enzymes, tolerant in the environment and fast growing.	(Chen *et al.,* 2022)
A. niger	Associated with otomycosis, skin infections, and lung disease, it has a broad spectrum of allergic diseases, has a worldwide distribution, and is a cause of hypersensitivity pneumonitis due to occupational exposure.	(Person *et al.,* 2010)
A. oryzae	It has been reported to infect immunocompromised people. It is susceptible to the main clinical antifungal drugs. *A. oryzae* strain ATCC 11866 also shares potentially harmful characteristics with the species *A. flavus*, which is a known plant pathogen and opportunistic animal pathogen, and is reported to cause sinus and eye infections in healthy humans and fatal lung disease and also systemic infection in susceptible individuals.	(He *et al.,* 2019; Kitagaki, 2021)
Neoscytalidium	Causing skin infections that mimic dermatophyte lesions, causing invasive or disseminated infections in transplant recipients or immunocompromised patients, patients from tropical areas present the highest risk.	(Garinet *et al.,* 2015)
R. stolonifer	Common fungus found in soil, is an agricultural pathogen that causes root rot, commonly found in tropical and subtropical regions.	(Baggio *et al.,* 2016)
C. tropicalis	Emerging pathogenic yeast, the most common cause of nosocomial candidemia, presents an acquired resistance to fluconazole with its consequent mortality rate.	(Kothavade *et al.,* 2010)
P. chrysogenum	Low pathogenicity, their infections are usually seen in immunocompromised patients, their diagnosis can be difficult, they are a generalized group that is found in the soil, decaying vegetation and in the air.	(Barcus *et al.,* 2005)
Trichosporon beigelii	Opportunistic pathogen, presents antifungal resistance, patients with disseminated trichosporonosis present a high mortality range.	(Erer *et al.,* 2000)
P. expansum	Blue mold, caused mainly by *P. expansum*, is a major threat to the global fruit industry, it produces a variety of mycotoxins that are detrimental to human health, the emergence of fungicide-resistant strains makes management options difficult.	(Luciano-Rosario *et al.,* 2020)
A. ustus	They rarely cause invasive disease in humans, the infections they cause may be of concern, as the organisms exhibit low susceptibility to multiple antifungal drugs.	(Panackal *et al.,* 2006)
P. purpurogenum	Plant pathogen, they produce rubratoxin B, a mycotoxin with anticarcinogenic properties, it has been recognized as a pathogen in few cases of human pulmonary infections in patients affected by hematological diseases.	(Zanatta *et al.,* 2006)
Curvalaria hominis	Dematiaceous saprophytic fungi, responsible for rhinosinusitis fungal allergies, are also the cause of intense inflammatory sinus allergies in immunocompromised individuals.	(Samaddar *et al.,* 2022), (Zapatero *et al.,* 2018)
A. tamarii	It is an emerging etiologic agent of human keratomycosis. This species has been reported in eyelid infections, onychomycosis, skin infections, wound infections, invasive sinonasal aspergillosis, nasal polyposis, and in respiratory tract samples.	(Homa *et al.,* 2019)

Figure 1 shows the number of isolates in each month for each well. Emphasizing the dry and wet season months. The dry season months are from December to April, and the wet season are from May to November (Jury *et al.,* 2007; Torres *et al.,* 2014). We observe that in the wet season there was a minimal increase in insulation. Although if the month of April 2018 and the month of April 2019 are observed, a decrease in species is noted. This can be due to several factors, including weather, dairy waste management, among others (Lotjonen *et al.,* 2020).

All the sampling wells were positive for filamentous fungi and yeasts (Figure 1). In total, 1759 CFUs were counted. Fungal counts ranged from 6 to 200 CFU/100 ml, averaging 74 to 54 CFU/100 ml per sampling well (Figure 2). The highest counts occurred in Well A in December 2018, January and February 2019 with 200 CFU/100 ml, followed by Well B and C in December 2018 and January 2019 with 200 CFU/100 ml. The lowest values occurred in well B in April 2018 with 6 CFU/100 ml.

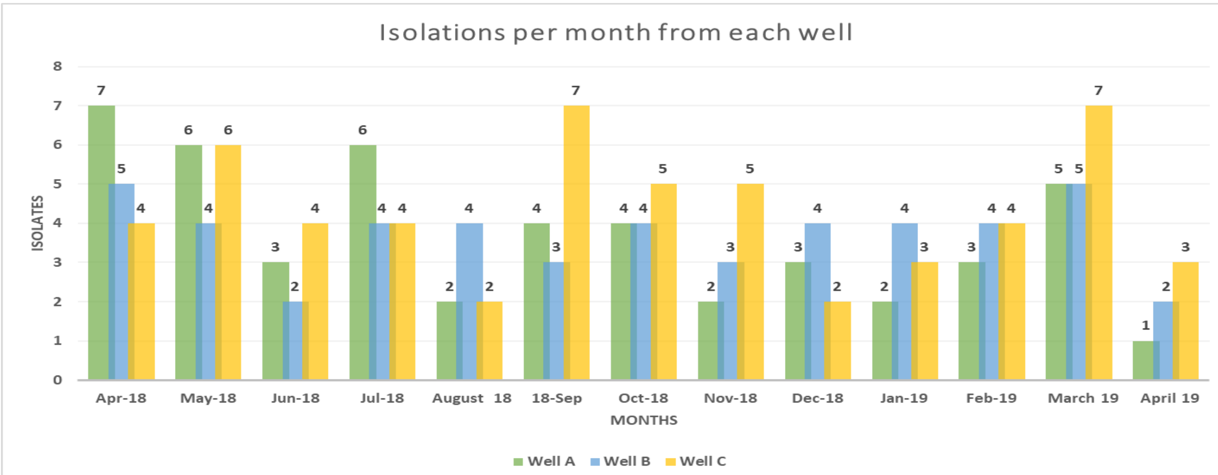
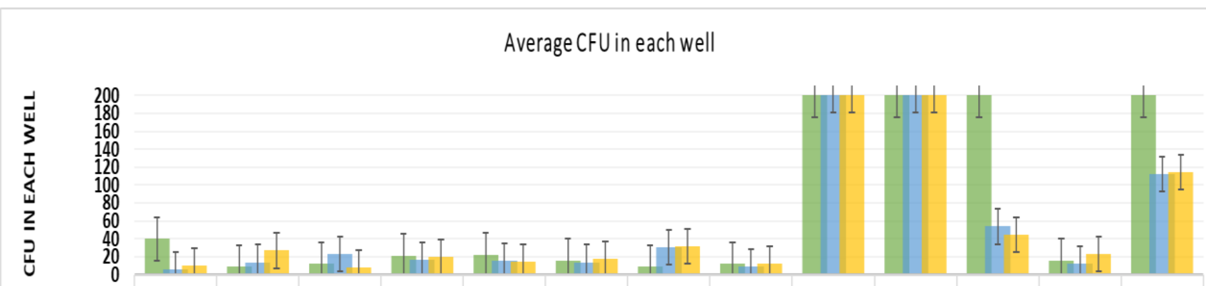


Fig. 1. Number of species by month and season.



	April 2018	May 2018	June 2018	July 2018	August 2018	September 2018	October 2018	November 2018	December 2018	January 2019	February 2019	March 2019	April 2019
Well A (CFU)	40	9	12	21	22	16	9	12	200	200	200	16	200
Well B (CFU)	6	14	23	17	16	14	31	9	200	200	54	12	112
Well C (CFU)	10	27	8	20	15	18	32	12	200	200	45	23	114

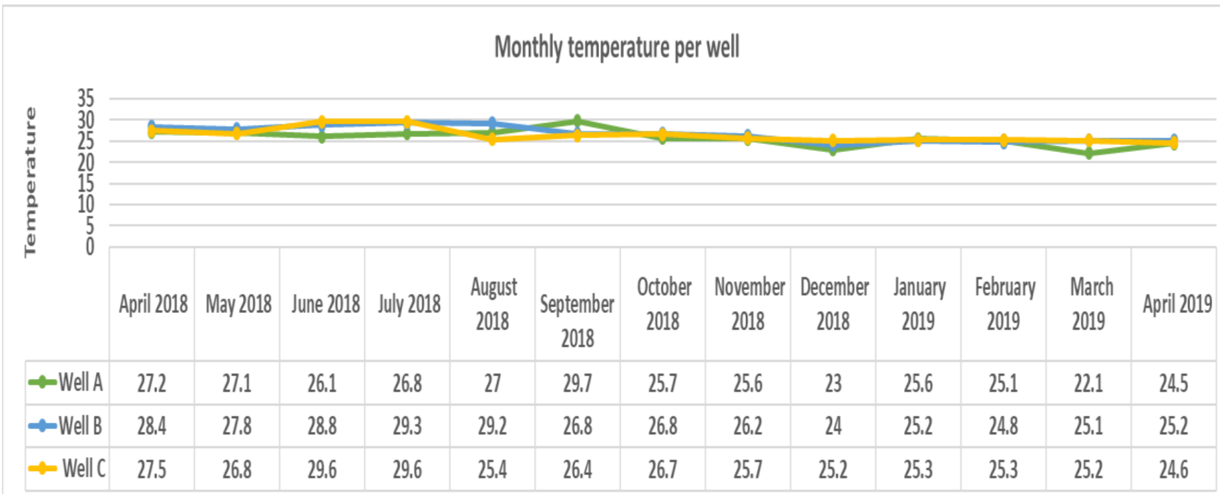
MONTH

Fig.2. Average CFU in the each well.

The temperature of the water samples, figure 3, indicates that the samples behaved quite uniformly. The water temperature varied from 22.1°C to 29.6°C, with the highest value in July

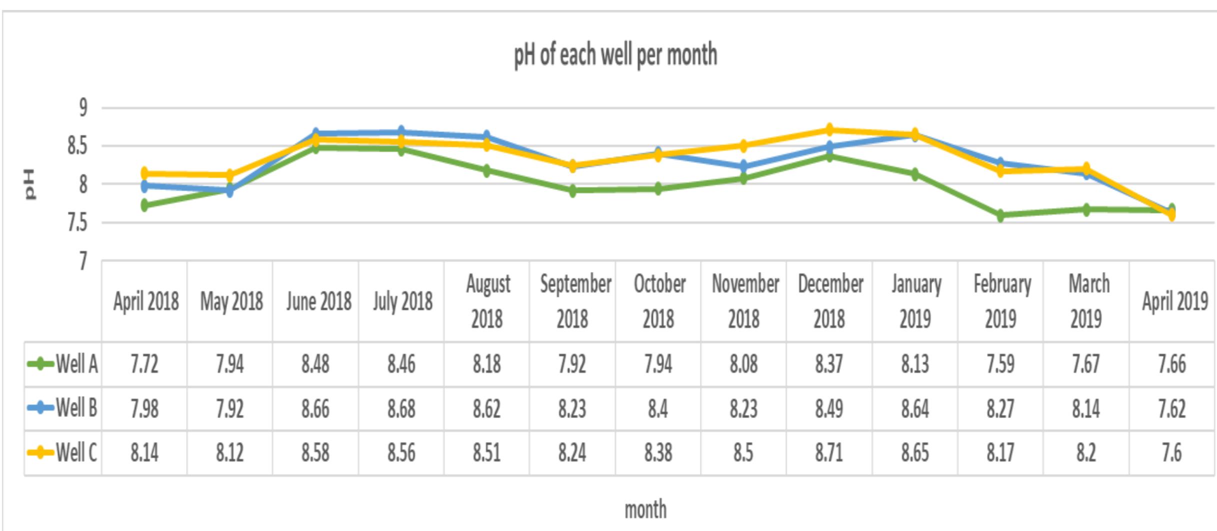
2018. The average temperature in well A was 25.8°C, well B was 26.7°C and well C was 26.4°C. The average value during the sampling period was 26.3 °C.

The pH varied between 7.6 to 8.7, in the three wells. Well A had an average of 7.69, Well B had 8.30 and Well C had 8.33 and the overall average was 8.11 (Figure 4). The highest value was observed in well B in June 2018 with a value of 8.66. These parameters were within the favorable range for fungal growth. In general, the range of physical-chemical parameters of the water evidences the growth of a wide diversity of fungi.



	April 2018	May 2018	June 2018	July 2018	August 2018	September 2018	October 2018	November 2018	December 2018	January 2019	February 2019	March 2019	April 2019
Well A	27.2	27.1	26.1	26.8	27	29.7	25.7	25.6	23	25.6	25.1	22.1	24.5
Well B	28.4	27.8	28.8	29.3	29.2	26.8	26.8	26.2	24	25.2	24.8	25.1	25.2
Well C	27.5	26.8	29.6	29.6	25.4	26.4	26.7	25.7	25.2	25.3	25.3	25.2	24.6

Fig. 3. Monthly temperature per well.



	April 2018	May 2018	June 2018	July 2018	August 2018	September 2018	October 2018	November 2018	December 2018	January 2019	February 2019	March 2019	April 2019
Well A	7.72	7.94	8.48	8.46	8.18	7.92	7.94	8.08	8.37	8.13	7.59	7.67	7.66
Well B	7.98	7.92	8.66	8.68	8.62	8.23	8.4	8.23	8.49	8.64	8.27	8.14	7.62
Well C	8.14	8.12	8.58	8.56	8.51	8.24	8.38	8.5	8.71	8.65	8.17	8.2	7.6

Fig. 4. pH of each well per month.

Al-Khatib et al. (2003), indicates that the main source of contamination in the waters are human or animal excreta, which reach humans through contaminated groundwater from wastewater, landfills, dairy farms or wastewater treatment stations. This causes serious health problems. The UN shows that diarrhea accounts for 80% of all diseases and more than a third of deaths in developing countries, caused by the consumption of contaminated water by people.

Most of these gastrointestinal infections can be transmitted through drinking water (Al-Khatib and Orabi, 2004). Filamentous fungi found in drinking water are common in water distribution systems and can be isolated in high concentrations (Hageskal, 2009). In Poland, the sample counts ranged from 20 to 500 CFU/100 ml (Grabinska-Loniewska *et al.,* 2007) and in Australia the counts were 33 and 97 CFU/100 ml for mains and reservoir water, respectively (Sammon *et al.,* 2010). In Egypt, Samah et al. (2014) tested groundwater for filamentous fungi and found 4 to 119 CFU/100 ml, while untreated groundwater found an average of 66 CFU/100 ml (Pereira *et al.,* 2009). Therefore, many of the colony growth values (CFU) are high and can represent a risk to the health of animals and sometimes to people who ingest the water from the wells.

Studies indicate that fungi survive and multiply in biofilm and sediment distribution systems, particularly at warmer temperatures or where flow is restricted (Kinsey *et al.,* 2003). A very important piece of research data indicates that the water that is stored and if it is in the dark for a long retention time and in stagnant areas, favors the increase of bacteria, fungi and the formation of biofilms. These biofilms help and are like a protection shield for microorganisms against disinfection and may be responsible for transferring fungi to most of the water (Siqueira *et al.,* 2011). Therefore, it indicates that the physical-chemical quality of the water, such as temperature, turbidity and TOC, can favor the growth and proliferation of fungi.

RECOMMENDATIONS

The livestock industry plays an important and direct role in waste management decisions. It is widely known that livestock production has a high potential for environmental degradation. As a result of this activity, a large volume of organic material, gases, fungi and bacteria is produced, which represents a risk factor for environmental contamination. High levels of effluents flow directly or indirectly to surface water and soil and from there to groundwater. Improved manure management on farms can prevent the dangerous flow of effluent into the water resource (Burton and Turner, 2003).

We can point out in our recommendations the use of biogas in the sustainable management of waste and the production of bioenergy obtained from waste from the livestock industry. One of the beneficial and advantageous processes in manure treatment is anaerobic digestion (Lantz *et al.,* 2007). Biogas is produced by bacterial conversion of organic matter under anaerobic conditions and is a mixture of carbon dioxide and the flammable methane gas. Biogas technology offers a unique set of benefits. Among them are that it is a sustainable source of energy, it benefits the environment, it provides a way to treat and reuse various wastes (human, animal, agricultural, industrial, state), it can be used for various energy services (heat, power, fuel for vehicles) and with additional treatment it could be possible to inject it into the natural gas network (Bond and Templeton, 2011).

The use of vermi-composting is also recommended. This is a modified composting system in which, instead of microorganisms, certain earthworms degrade manure and other waste through feeding and disposal (Echevarría, 2020). This method is more cost effective than other technologies used to transform manure. Other advantages that vermi-composting provides are: it improves aeration, texture and water retention capacity in the soil, it improves the state of nutrients of both the plant and the soil, it promotes root growth and absorption of nutrients and has no adverse effect (Domínguez and Edwards, 2011).

Storage of animal waste is recommended as a practical strategy with other advantages in nutrient management and protection of soil and water quality. Containment strategies include preventing spills and seepage into ponds, controlling surface runoff, and limiting sediment erosion and transport from farms. Surface runoff can be constrained by better manure collection and storage capacity allowing manure to be applied to land only when crop and water demand is high. Long-term manure storage offers benefits in terms of containment and can result in a lower prevalence of contaminating residues (Chee-Sanford *et al.,* 2012).

The efficient use of waste from the livestock industry requires proper disposal and a return of nutrients to the soil without causing contamination and the spread of pathogens that can migrate to underground water supplies. Good waste management has to include the collection, transport, treatment and disposal of waste, together with optimal routine monitoring and a genuine commitment to the application of established regulations. An adequate management in the management of waste in the livestock industry is necessary to prevent the contamination problems that it produces from continuing to increase.

CONCLUSION

The present study advances in the understanding of contamination by fungi and yeasts in water systems. The average fungal colonies of the three wells fluctuated from 6 to 200 CFU, pathogenic species to humans were found. Among the isolated fungi, *A. nidulans, A. oryzae, A. terreus, A. niger, A. flavus, A. ochraceus, A. tamarii, A. ustus, R. stolonifer, C. tropicalis, C. paripsilisis, C. rugosa, C. purpurogenum, P. chrysogenum, P. expansum, Curvularia homins* and *Trichosporon beigelii* from water samples from the 3 wells. The temperature parameters were from 22.1°C to 29.6°C, while the pH was from 7.62 to 8.66. It is important to have a control system in place to prevent the growth of these fungi and this must be checked regularly; therefore, chlorination conditions must be managed well. The results of this study are important since they determine the mycological quality of the water in the wells of the dairy farms, as well as the need for adequate measures to prevent fungal growth and the development of the necessary strategies.

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CONFLICT OF INTEREST

The author declares that this article content has no conflict of interest.

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